

SUPPLEMENTARY INFORMATION

**Novel Roles for ERK5 and Cofilin as Critical Mediators Linking Estrogen Receptor α -
Driven Transcription, Actin Reorganization and Invasiveness in Breast Cancer**

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Supplementary Figure Legends

Supplementary Figure 1. Verification of pERK5 recruitment to binding sites identified by

ChIP-Seq in MCF-7 cells. ChIP-qPCR of pERK5 recruitment to regulatory regions of various E2-regulated genes was conducted. MCF-7 cells were treated with vehicle or 10 nM E2 for 45 min. After formaldehyde cross-linking and sonication, pERK5/DNA complexes were immunoprecipitated. Immunoprecipitated DNA levels were measured by qPCR and % input was calculated. The results (mean \pm SD) are from three independent experiments.

Supplementary Figure 2. Impact of ERK5 depletion on gene regulations in estradiol treated cells.

(a) Comparison of number of 17 β -estradiol (E2) regulated genes after 24 h of ligand treatment in MCF-7 cells. Genespring Venn Diagram tool was used to compare overlap between genes regulated greater than or equal to 1.8-fold with FDR of 0.01 in each background, i.e. cells treated with control siRNA or ERK5 siRNA for 72 h before hormone treatment for 24h.

(b) Scatter plots for gene regulations in the different cell backgrounds: scatter plots of E2-regulated genes in each cell background were generated using the Genespring scatterplot tool. The top two panels show how genes that are regulated by E2 in siCtrl transfected cells are also being regulated in siERK5 transfected cells. The bottom two panels show how genes that are regulated by E2 in siERK5 transfected cells are also being regulated in siCtrl transfected cells. E2-upregulated genes are shown in red and E2-downregulated genes in blue.

Supplementary Figure 3. Loss of pERK5 localization to the nucleus in the absence of ER α .

MCF-7, BT474 and T47D cells were transfected with 20 nM siCtrl or siER α for 72 h and then treated with 10 nM of E2 for 45 min and immunofluorescence microscopy was performed with antibody specific to pERK5. F-actin was stained and visualized with Alexa568-phalloidin. Nuclei were stained and visualized with DAPI.

Supplementary Figure 4. Loss of ERK5, pERK5 and CFL1 localization to the nucleus in the absence of ER α activity and ERK5 activity. MCF-7 cells were treated with Ctrl (0.1% EtOH), 1 μ M of the antiestrogen Fulvestrant or 1 μ M XMD8-92 (ERK5 inhibitor) for 24 h and immunofluorescence microscopy was performed with an antibody specific to ERK5 (Upper Left) and ER α , pERK5 (upper right) or CFL1 (lower left). F-actin was stained and visualized with Alexa568-phalloidin. Nuclei were stained and visualized with DAPI.

Supplementary Figure 5. ERK5 regulates transcription after estradiol treatment of MCF-7 cells. Impact of ERK5-DN expression on recruitment of factors associated with transcription. MCF-7 cells were infected with AdCMV (Ctrl) or AdERK5-DN for 24h and then treated with control vehicle or 10 nM E2 for 45 min. Factor/DNA complexes were immunoprecipitated using specific antibody to PAF1, SPT5, RNA Pol II, CyclinT1 or WDR61. Immunoprecipitated DNA levels were measured by qPCR and % input was calculated. Values are the mean \pm SD of three independent experiments.

Supplementary Figure 6. Intracellular localization of pERK5 after expression of ER α in three ER α -negative breast cancer cell lines. MDA-MB-468, MDA-MB-453 and SKBR3 cells were infected with control AdCMV or AdER α for 96 h and immunofluorescence microscopy

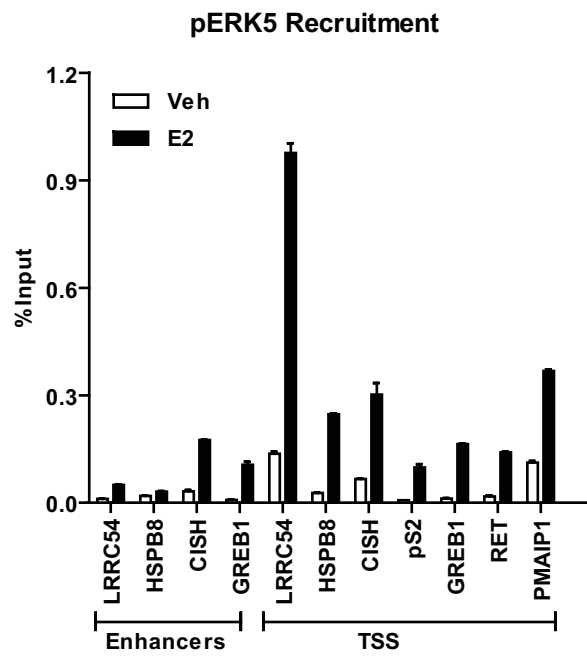
was performed with antibodies specific to pERK5 and ER α . Nuclei were stained and visualized with DAPI.

Supplementary Figure 7. Colocalization of pERK5 and F-actin after ER α introduction into ER α -negative cells. MDA-MB-468 cells were infected with AdCMV or AdER α for 96 h and immunofluorescence microscopy was performed with an antibody specific to pERK5. F-actin was stained and visualized with Alexa568-phalloidin. Nuclei were stained and visualized with DAPI.

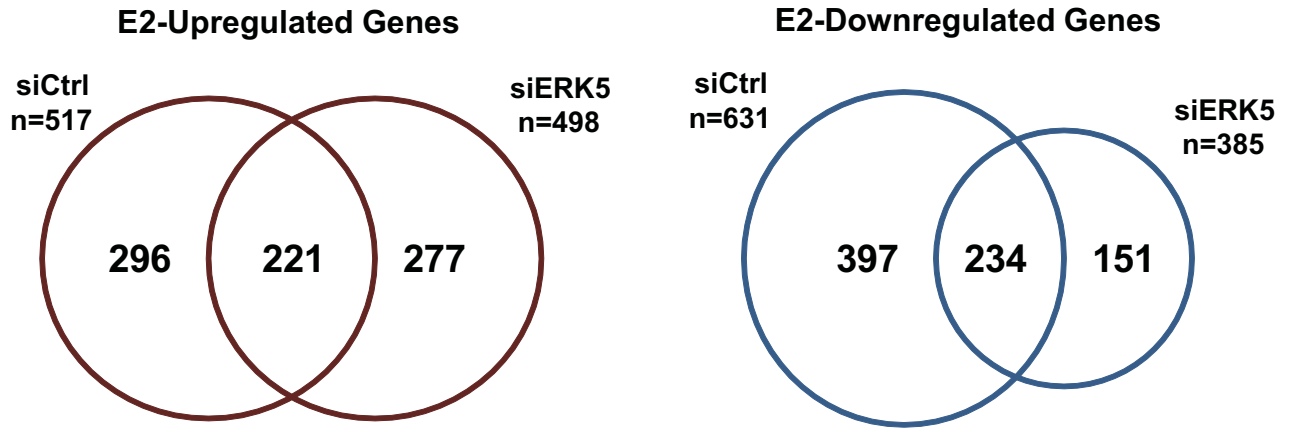
Supplementary Table Legends

Table S1. GO Term Enrichment Analysis for Genes whose Regulation by E2 was affected by ERK5 knockdown.

Table S2. List of E2-regulated signature genes requiring chromatin recruitment of ERK5 for their hormonal regulation.

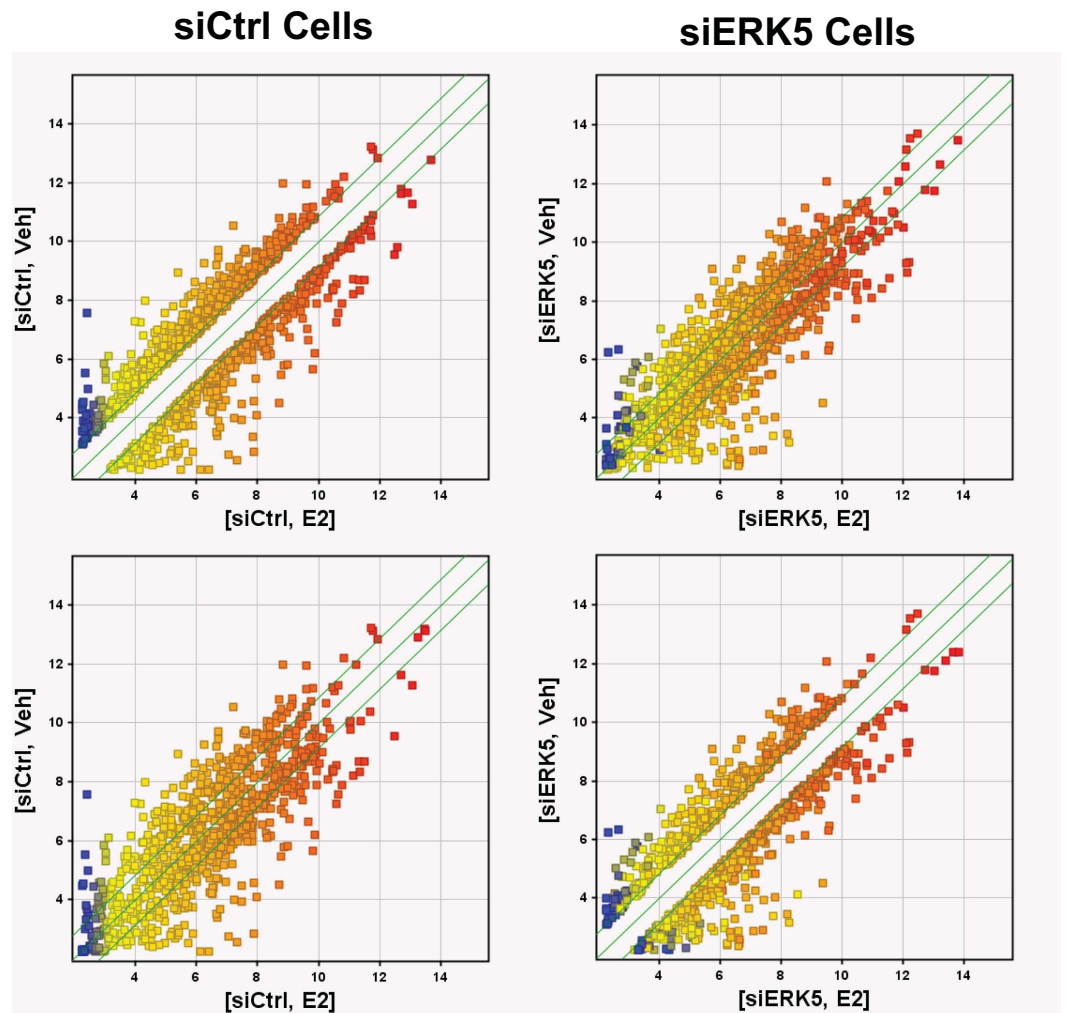


Supplementary Figure 1

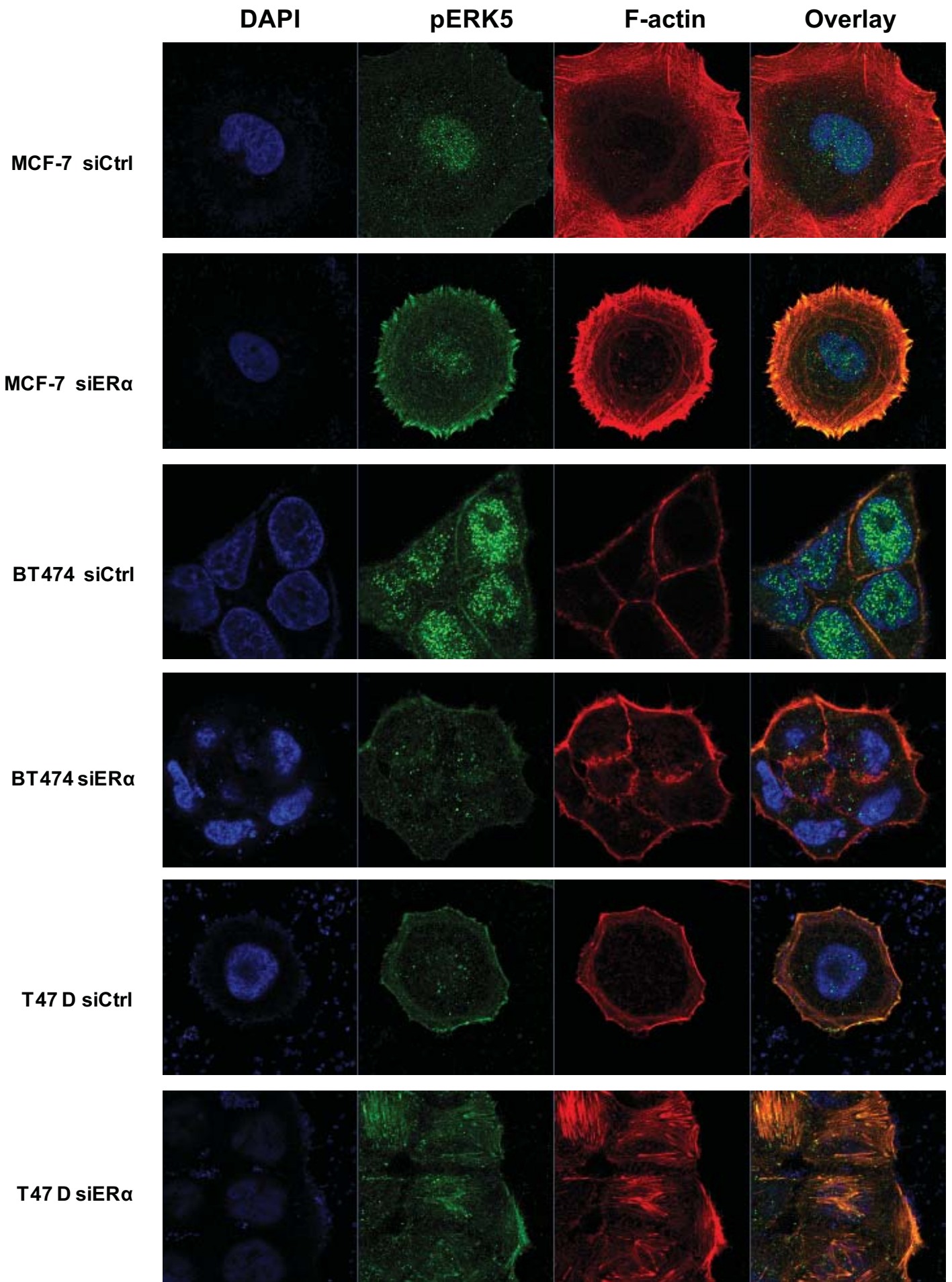
A**B**

**E2-Regulated Genes
in siCtrl Cells**

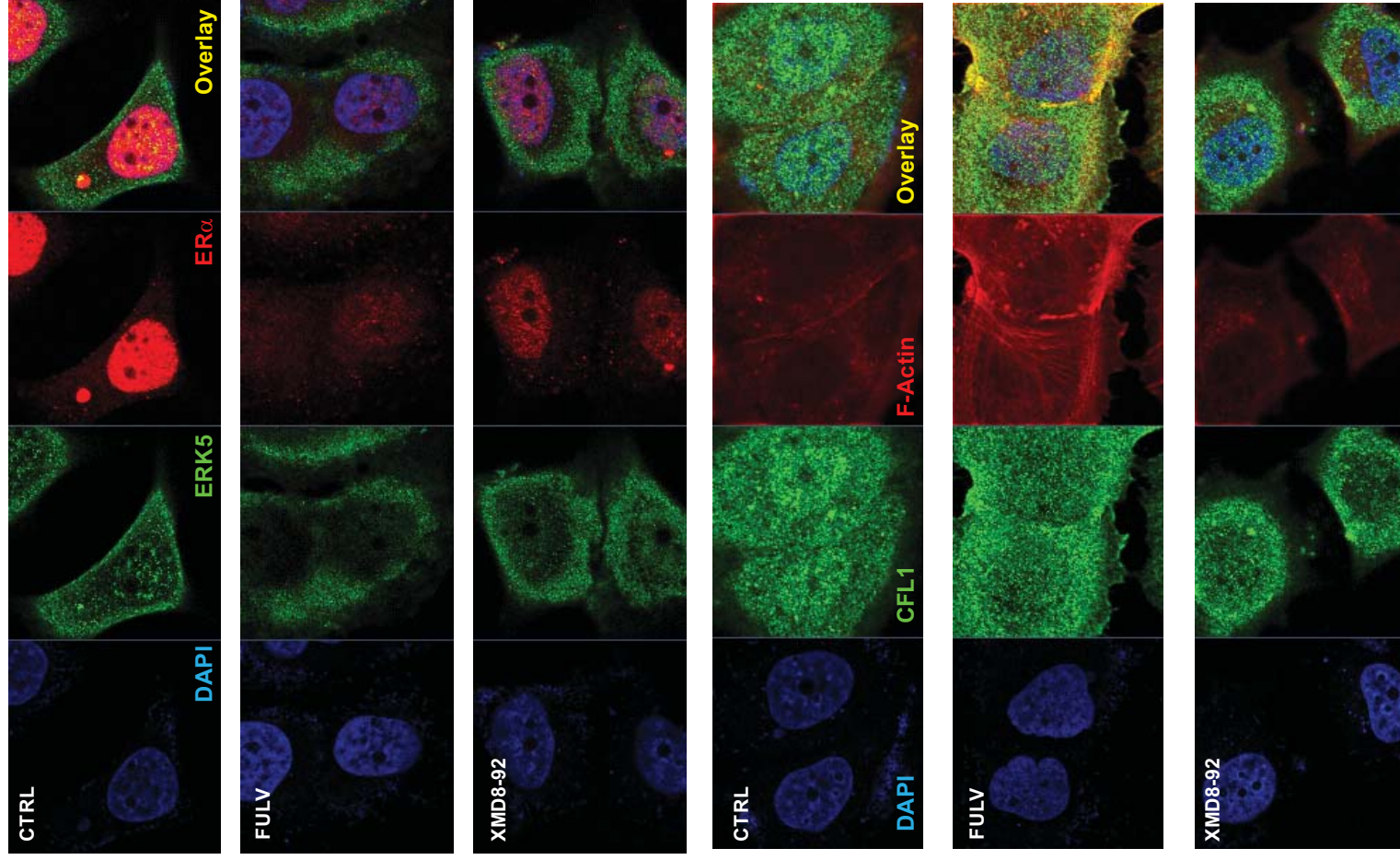
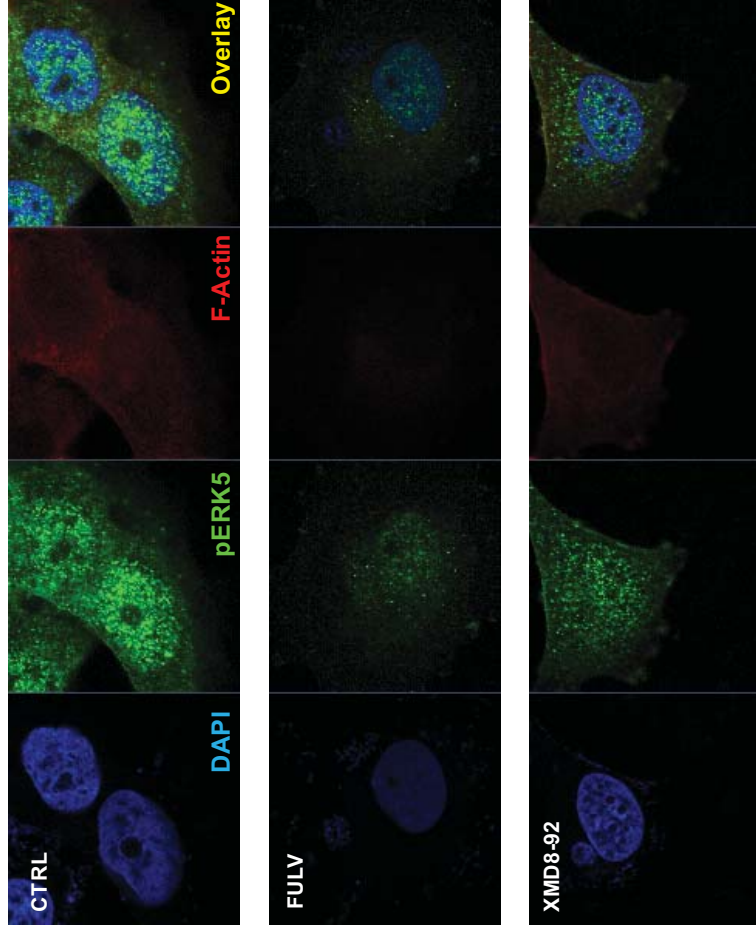
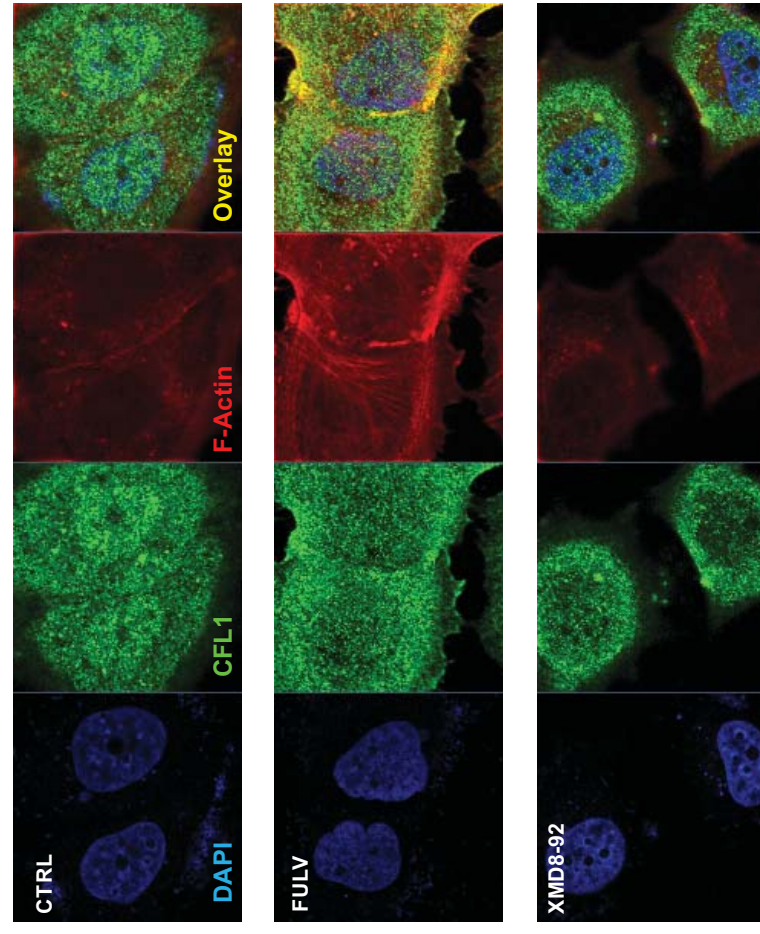
**E2-Regulated Genes
in siERK5 Cells**



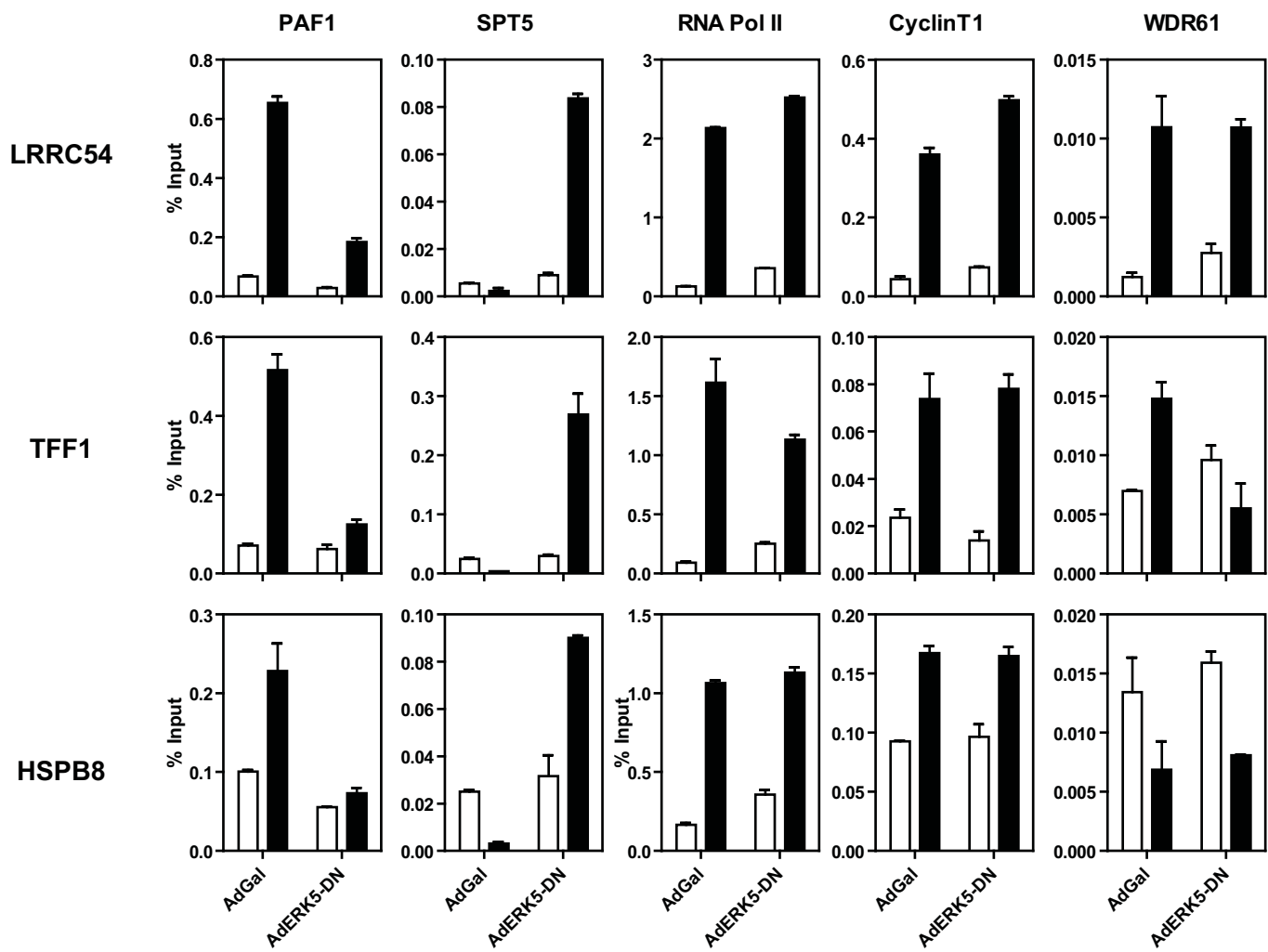
Supplementary Figure 2



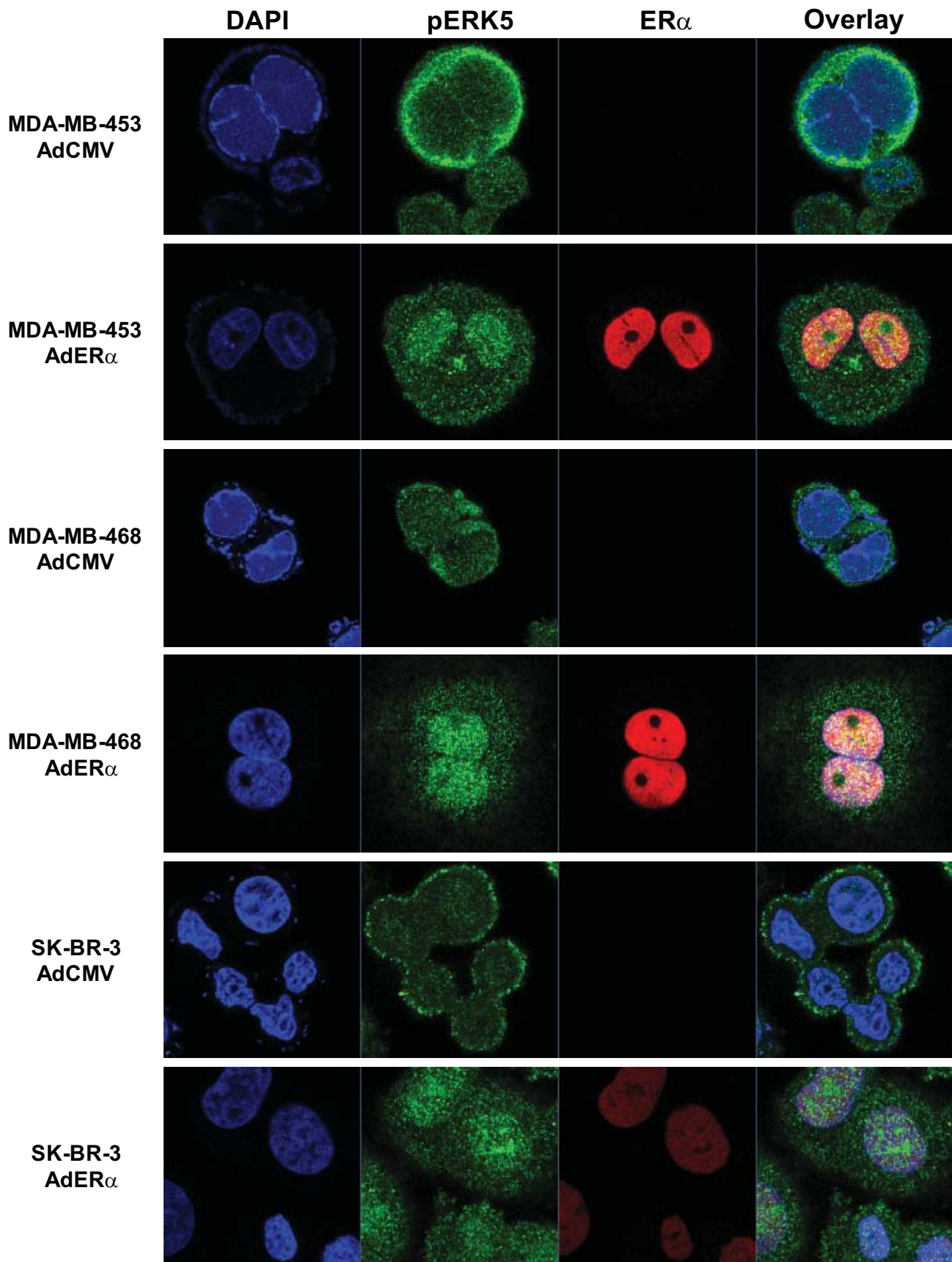
Supplementary Figure 3

A**B****C**

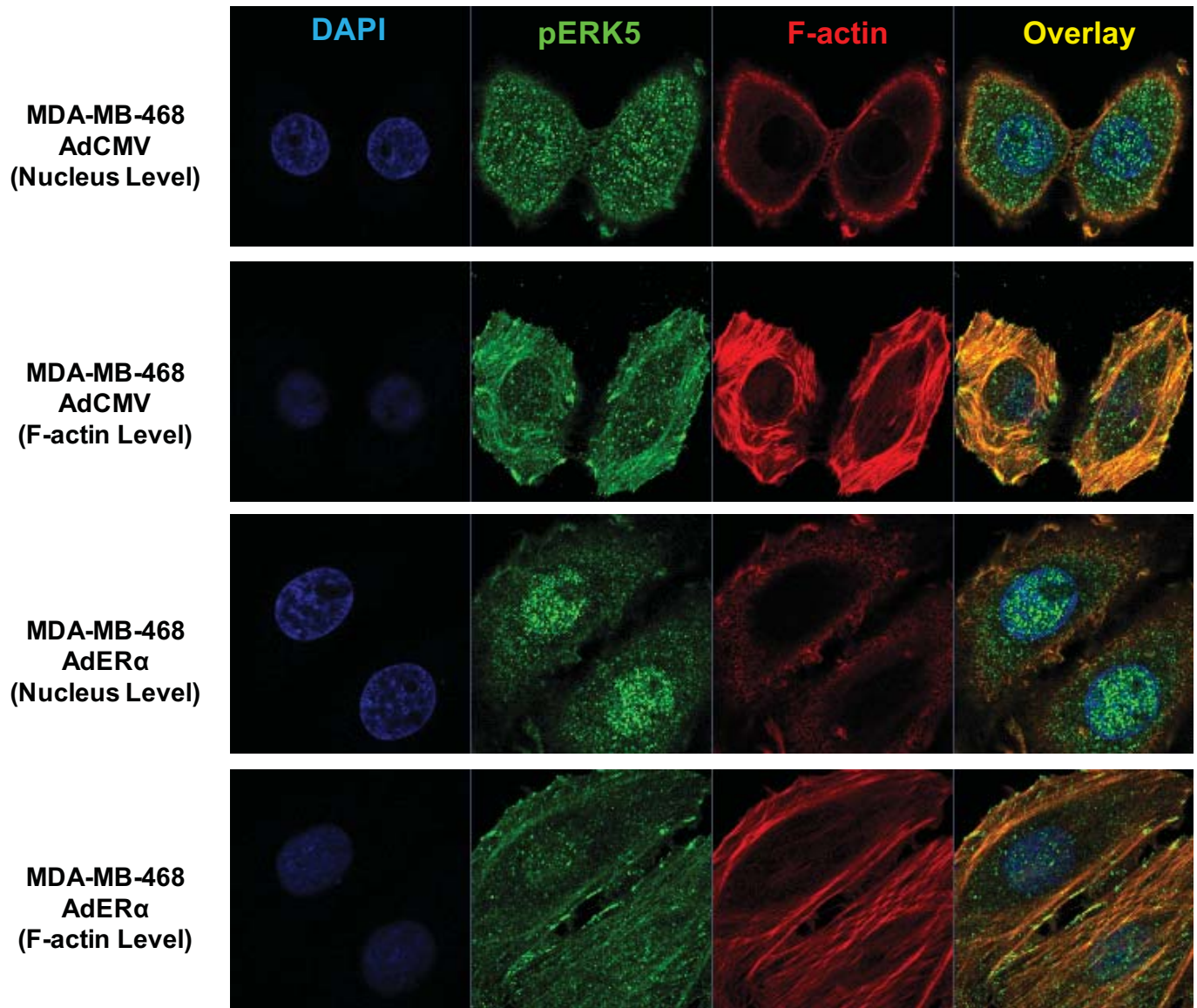
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7

Table S1. GO term Enrichment Analysis for Genes whose Regulation by E2 Stimulation was affected by ERK5 knockdown

Enrichment by Pathway Maps for E2 stimulated genes which are blocked by ERK5 knockdown

#	Maps	Total	pValue	Min FDR
1	Cell cycle Start of DNA replication in early S phase	32	0.000E+00	0
2	Cell cycle Role of SCF complex in cell cycle regulation	29	4.926E-08	3.0295E-06
3	Cell cycle Role of APC in cell cycle regulation	32	3.045E-06	0.000124853
4	DNA damage ATM / ATR regulation of G2 / M checkpoint	26	3.413E-05	0.001049494
5	Cell cycle Transition and termination of DNA replication	28	4.624E-05	0.001137382
6	DNA damage ATM/ATR regulation of G1/S checkpoint	32	7.945E-05	0.001579904
7	Cell cycle ESR1 regulation of G1/S transition	33	8.991E-05	0.001579904
8	Apoptosis and survival DNA-damage-induced apoptosis	15	1.584E-04	0.002434862
9	DNA damage Role of Brca1 and Brca2 in DNA repair	30	1.307E-03	0.017863367
10	DNA damage DNA-damage-induced responses	9	1.826E-03	0.022465943

Enrichment by Pathway Maps for E2 stimulated genes which are enhanced by ERK5 knockdown

#	Maps	Total	pValue	Min FDR
1	Immune response HSP60 and HSP70/TLR signaling pathway	54	2.324E-06	0.000579236
2	Immune response IL-17 signaling pathways	60	4.355E-06	0.000579236
3	Apoptosis and survival Apoptotic TNF-family pathways	42	1.216E-05	0.001078462
4	Immune response MIF-mediated glucocorticoid regulation	22	1.707E-05	0.001135078
5	Immune response TLR signaling pathways	54	4.228E-05	0.002249381
6	Bacterial infections in CF airways	58	5.991E-05	0.002473333
7	Immune response TREM1 signaling pathway	59	6.509E-05	0.002473333
8	Immune response CD40 signaling	65	1.038E-04	0.003452811
9	Immune response Gastrin in inflammatory response	69	1.382E-04	0.004083634
10	Signal transduction AKT signaling	43	2.568E-04	0.006830088

Enrichment by Pathway Maps for E2 repressed genes which are blocked by ERK5 knockdown

#	Maps	Total	pValue	Min FDR
1	Development ERK5 in cell proliferation and neuronal survival	23	4.153E-04	0.116685748
2	Apoptosis and survival NGF activation of NF-kB	29	8.335E-04	0.117111448
3	Transcription Receptor-mediated HIF regulation	39	1.992E-03	0.150561121
4	Apoptosis and survival Ceramides signaling pathway	40	2.143E-03	0.150561121
5	Development PIP3 signaling in cardiac myocytes	47	3.407E-03	0.159554553
6	Development HGF signaling pathway	47	3.407E-03	0.159554553
7	G-protein signaling Proinsulin C-peptide signaling	52	4.539E-03	0.168220616
8	Development WNT signaling pathway_Part 2	53	4.789E-03	0.168220616

9	Cytoskeleton remodeling_TGF_WNT and cytoskeletal remodeling	111	5.648E-03	0.17635468
10	Development_WNT signaling pathway. Part 1. Degradation of beta-cate	19	6.561E-03	0.184377933

Enrichment by Pathway Maps for E2 repressed genes which are enhanced by ERK5 knockdown

#	Maps	Total	pValue	Min FDR
1	Histidine-glutamate-glutamine metabolism	95	1.032E-02	0.124836896
2	Immune response_Sialic-acid receptors (Siglecs) signaling	12	1.962E-02	0.124836896
3	Cytoskeleton remodeling_CDC42 in cellular processes	22	3.569E-02	0.124836896
4	Apoptosis and survival_Anti-apoptotic TNFs/NF-kB/IAP pathway	27	4.363E-02	0.124836896
5	Development_Thrombospondin-1 signaling	28	4.521E-02	0.124836896
6	G-protein signaling_RAC1 in cellular process	35	5.622E-02	0.124836896
7	Regulation of metabolism_Bile acids regulation of glucose and lipid meta	37	5.934E-02	0.124836896
8	Cell adhesion_Cell-matrix glycoconjugates	38	6.090E-02	0.124836896
9	Apoptosis and survival_APRIL and BAF signaling	39	6.245E-02	0.124836896
10	Immune response_Alternative complement pathway	39	6.245E-02	0.124836896

Table S2.**List of E2-regulated signature genes requiring chromatin recruitment of ERK5 for their hormonal regulation**

Gene Name	Gene Symbol
S-phase kinase-associated protein 2 (p45)	SKP2
exonuclease 1	EXO1
glycyl-tRNA synthetase	GARS
cell division cycle 45 homolog (<i>S. cerevisiae</i>)	CDC45
CHK1 checkpoint homolog (<i>S. pombe</i>)	CHEK1
minichromosome maintenance complex component 10	MCM10
solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	SLC7A5
polymerase (DNA directed), theta	POLQ
CDC28 protein kinase regulatory subunit 1B	CKS1B
polo-like kinase 4 (<i>Drosophila</i>)	PLK4
serine hydroxymethyltransferase 2 (mitochondrial)	SHMT2
RAD54-like (<i>S. cerevisiae</i>)	RAD54L
centromere protein N	CENPN
ribonuclease P/MRP 40kDa subunit	RPP40
GIN5 complex subunit 3 (Psf3 homolog)	GIN53
spindle and kinetochore associated complex subunit 1	SKA1
Bloom syndrome, RecQ helicase-like	BLM
tyrosyl-tRNA synthetase	YARS
phosphoserine aminotransferase 1	PSAT1
minichromosome maintenance complex component 2	MCM2
chromatin licensing and DNA replication factor 1	CDT1
protein kinase, membrane associated tyrosine/threonine 1	PKMYT1
flap structure-specific endonuclease 1	FEN1
excision repair cross-complementing rodent repair deficiency, complementation group 6-like	ERCC6L
methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methylenetetrahydrofolate cyclohydrolase	MTHFD2
RAD51 associated protein 1	RAD51AP1
argininosuccinate synthase 1	ASS1
methionyl-tRNA synthetase	MARS
minichromosome maintenance complex component 4	MCM4
minichromosome maintenance complex component 6	MCM6
cell division cycle 25 homolog A (<i>S. pombe</i>)	CDC25A
chloride channel CLIC-like 1	CLCC1
defective in sister chromatid cohesion 1 homolog (<i>S. cerevisiae</i>)	DSCC1

nei endonuclease VIII-like 3 (E. coli)	NEIL3
RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae)	RAD51
asparagine synthetase (glutamine-hydrolyzing)	ASNS
suppressor of variegation 3-9 homolog 1 (Drosophila)	SUV39H1
nucleolar and coiled-body phosphoprotein 1	NOLC1
replication factor C (activator 1) 3, 38kDa	RFC3
minichromosome maintenance complex component 3	MCM3
calcyclin binding protein	CACYBP
centromere protein M	CENPM
minichromosome maintenance complex component 5	MCM5
eukaryotic translation initiation factor 4E binding protein 1	EIF4EBP1
phosphoserine phosphatase	PSPH
translocase of inner mitochondrial membrane 8 homolog A (yeast)	TIMM8A
chromatin assembly factor 1, subunit B (p60)	CHAF1B
uracil-DNA glycosylase	UNG
ASF1 anti-silencing function 1 homolog B (S. cerevisiae)	ASF1B
interleukin 1 receptor accessory protein	IL1RAP
solute carrier family 7 (cationic amino acid transporter, y+ system), member 1	SLC7A1
malic enzyme 2, NAD(+)-dependent, mitochondrial	ME2
REX2, RNA exonuclease 2 homolog (S. cerevisiae)	REXO2
cysteinyI-tRNA synthetase	CARS
TIMELESS interacting protein	TIPIN
nuclear autoantigenic sperm protein (histone-binding)	NASP
Fanconi anemia, complementation group G	FANCG
polymerase (DNA directed), delta 1, catalytic subunit 125kDa	POLD1
activating transcription factor 3	ATF3
CCAAT/enhancer binding protein (C/EBP), gamma	CEBPG
GIN5 complex subunit 4 (Sld5 homolog)	GIN54
activating transcription factor 4 (tax-responsive enhancer element B67)	ATF4
myosin XIX	MYO19
transmembrane protein 194A	TMEM194A
solute carrier family 1 (neutral amino acid transporter), member 5	SLC1A5
denticleless homolog (Drosophila)	DTL
chromatin assembly factor 1, subunit A (p150)	CHAF1A
thymopoietin	TMPO
translocase of inner mitochondrial membrane 44 homolog (yeast)	TIMM44

neurofibromin 2 (merlin)	NF2
transmembrane protein 38B	TMEM38B
polymerase (DNA directed), epsilon 2 (p59 subunit)	POLE2
cell division cycle 6 homolog (S. cerevisiae)	CDC6
replication factor C (activator 1) 5, 36.5kDa	RFC5
methyltransferase like 1	METTL1
tripeptidyl peptidase II	TPP2
branched chain amino-acid transaminase 1, cytosolic	BCAT1
centromere protein Q	CENPQ
embryonic ectoderm development	EED
EMG1 nucleolar protein homolog (S. cerevisiae)	EMG1
DNA cross-link repair 1B (PSO2 homolog, S. cerevisiae)	DCLRE1B
leucine zipper-EF-hand containing transmembrane protein 1	LETM1
primase, DNA, polypeptide 1 (49kDa)	PRIM1
non-SMC condensin I complex, subunit H	NCAPH
breast cancer 1, early onset	BRCA1
WD repeat and HMG-box DNA binding protein 1	WDHD1
nuclear protein, transcriptional regulator, 1	NUPR1
GIN5 complex subunit 2 (Psf2 homolog)	GIN5
protein tyrosine phosphatase, receptor type, G	PTPRG
retinoblastoma binding protein 8	RBBP8
breast cancer 2, early onset	BRCA2
Scm-like with four mbt domains 1	SFMBT1
GDNF family receptor alpha 1	GFRA1
dedicator of cytokinesis 4	DOCK4
zinc finger protein 232	ZNF232
YEATS domain containing 4	YEATS4
neurexin 3	NRXN3
solute carrier family 7, (cationic amino acid transporter, y+ system) member 11	SLC7A11
laminin, alpha 3	LAMA3
zinc finger protein 174	ZNF174
CD44 molecule (Indian blood group)	CD44
notchless homolog 1 (Drosophila)	NLE1
zinc finger protein 33B	ZNF33B
inhibitor of growth family, member 2	ING2
basic helix-loop-helix family, member e41	BHLHE41

phosphoenolpyruvate carboxykinase 2 (mitochondrial)	PCK2
PDX1 C-terminal inhibiting factor 1	PCIF1
tetraspanin 5	TSPAN5
ADP-ribosylation factor 3	ARF3
glutamate decarboxylase 1 (brain, 67kDa)	GAD1
B9 protein domain 1	B9D1
solute carrier family 25 (mitochondrial oxodicarboxylate carrier), member 21	SLC25A21
solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	SLC1A4
solute carrier family 22 (organic cation/ergothioneine transporter), member 4	SLC22A4
stanniocalcin 2	STC2
progesterone receptor	PGR
BCL2-associated athanogene 2	BAG2
chromosome 14 open reading frame 139	C14orf139
eukaryotic translation initiation factor 2B, subunit 3 gamma, 58kDa	EIF2B3